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13. ABSTRACT (Maximum 200 Words)

African American men are at greater risk for both developing and dying from prostate cancer compared to white men. The reason for this disparity is likely due to a number of factors including environmental exposures and genetic factors. The Flint Men's Health Study (FMHS) was established in 1995 as a population-based case-control study of African American men aged 40-79 residing in Genesee County, Michigan. The initial sample consisted of 730 men who completed an extensive in-home interview consisting of potential risk factors for prostate cancer; medical history; and demographic information. From this initial cohort, 431 men provided a blood sample and 369 men who were determined to be free of cancer completed a comprehensive urologic examination. Additionally, 119 cases of prostate cancer have been identified from the same study population. Studies have suggested a role for hormones and genetics in prostate cancer incidence. However, these studies have been completed in white populations and results have been conflicting. The objective of this study is to use a set of previously collected serum and DNA samples from a population-based study of African American men to more clearly delineate the potential role(s) of selected hormones and growth factors in prostate cancer development.

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INTRODUCTION

Prostate cancer is the most common cancer and the second leading cause of cancer deaths in U.S. men. African American men are at greater risk for both developing and dying from prostate cancer compared to white men.(1) Research based on racial differences in prostate cancer has resulted in important discoveries that have just begun to unravel the complex biologic mechanisms for prostate cancer. The paucity of data on prostate cancer from population-based samples of African American men highlights a gap in our current understanding of the disease. The goal of this proposed study is to determine whether or not there are differences in 1) various circulating hormone and growth factor levels and 2) the prevalence of genetic polymorphisms between African American men with prostate cancer and those free of disease. We will also measure the associations between genetic polymorphisms and circulating hormone levels and their subsequent impact on prostate cancer risk in African American men. This information will enable us to better understand the role of hormonal and genetic risk factors in the disease process. We will make use of the unique opportunity presented by the availability of an ongoing epidemiologic study of community-dwelling African American men: the Flint Men's Health Study. We will accomplish this through the following Specific Aims:

Specific Aim 1: To evaluate the associations between *circulating hormone* and *growth factor* levels and *prostate cancer* using samples from a population-based cohort of African American men (Table 1).

Specific Aim 2: To evaluate the associations between *genetic polymorphisms* and *prostate cancer* using samples from a population-based cohort of African American men.

a. To evaluate differences in the prevalence of common *genetic polymorphisms* (Table 3) between men diagnosed with prostate cancer and disease free controls.
b. To evaluate differences in the prevalence of common *genetic polymorphisms* (Table 3) between men diagnosed with various stage and grade prostate cancer.

Specific Aim 3: To measure the associations between genetic polymorphisms and circulating hormone levels and their subsequent impact on prostate cancer risk in African American men.

The completion of these aims will lead to new insights into differences and/or similarities in the prevalence of hormonal and genetic correlates of prostate cancer between cases and controls. These insights will provide the direction for the next sets of studies to better define the etiology of prostate cancer in African American men, its natural history and clinic course, as well as potential targets for intervention.

BODY

The following include research accomplishments associated with tasks outlined in our approved Statement of Work.

Task 1. **To perform laboratory assays for the following hormones:**

Months 1-12
Total Testosterone
Free Testosterone
Androstenedione
Dihydroepiandrosterone sulfate
Serum hormone binding globulin
Androstenediol glucuronide
Sodium

Months 12-24
Estradiol
Estrone Sulfate
Insulin-like Growth Factor-1
Insulin-like Growth Factor Binding Protein-3

This task has been completed and included sending aliquots of over 450 serums to the appropriate laboratories, entering the data into spreadsheets, and data cleansing. Specifically, circulating hormone levels listed above were quantified by performing laboratory assays at the University of Michigan Medical Center Reproductive Sciences and Clinical Chemistry Labs. Total IGF-1, IGFBP-3 and AAG were measured by a commercially available enzyme-linked immunosorbent assays (ELISA) (Diagnostic Systems Laboratory, Webster, Texas) in Dr. Jaffe's laboratory. Inter-assay and intra-assay coefficients of variation were as follows: IGF-1: 4%,6%; IGFBP-3: 6%,9%; and AG: 5% and 11%. All other hormones were measured using commercially available chemiluminescent immunoassays (Bayer Diagnostics, Pittsburgh, PA) Inter-assay and intra-assay coefficients of variation, respectively, were as follows: DHEAS:18.14%, 12.53%; TT:8.68%, 6.82%; FT:6.5%, 7.3%; SHBG:18.95%, 10.31%; Androstenedione:11.6%, 6 %; E2:10.21%, 6.375%; and Estrone:10.27%, 10.93%.

Task 2. **To perform DNA analyses to examine the following genotypes:**

Months 1-12	Months 12-24
LHB	CYP19
HSD3B2	CYP3A4
CYP17	IGF1
HSD17B2	

We had initially planned to perform our SNP assays using either ABI PRISM® 7700 Sequence Detection System which uses TaqMan® assays or an ABI 3100 PRISM®Genetic Analyzer which employs a 16 capillary electrophoresis system. However the University of Michigan Comprehensive Cancer Center cDNA Core recently purchased the ABI PRISM® 7900 Sequence Detection System which has the capacity to perform high-throughput SNP detection using 384-well plates. We have performed a number of pilot experiments and have learned that SNP assays using the ABI 7900 are very sensitive to DNA concentration. We have optimized our assays and the failure rate is now less than 5%. This new genotyping platform has the reduced the estimated cost per genotype from $2.00 to less than $0.50. This will allow us to perform additional genotyping in years 1-2 of the grant.

For year 1, we are working on completion of proposed assays above as well as selection for new assays. Assays have been performed for SNPs in the following genes: *AR, HSD3B2, HSD17B, CYP17, CYP19, CYP3A5, SRD5A2.* Genotying will continue thru year 2, however, data analyses and presentations on completed genotypes have already begun.

Task 3. **Interim statistical analyses of data obtained from hormone assays and DNA will be performed periodically as assays are performed. (Months 1-24)**

For year 1, we completed preliminary statistical analyses of proposed serum hormone data and Preliminary statistical analyses of genotyping data are underway. Additionally, more comprehensive analyses and manuscript preparation for various serum hormone data have already begun.

Task 4. **Final analyses and report writing (Months 24-36)**

a. Final analyses of data from hormone assays and genetic polymorphisms will be performed.
b. Final manuscripts will be prepared and submitted.

This task has not yet been started.

KEY RESEARCH ACCOMPLISHMENTS

- Completion of all proposed serum hormone assays
- Completion of genotypes in 7 genes
- Selection of new SNPs underway
- Completion of interim statistical analyses on serum hormone and genotype data

REPORTABLE OUTCOMES

In this first year of funding, we have made significant progress towards completing laboratory studies required to carry out analyses described in Specific Aims 1, 2 and 3. Table 1 reports initial comparisons of mean serum hormone concentrations by prostate cancer status. Additional analyses will be performed to examine potential confounders in the relationship between these circulating hormones and the risk of prostate cancer and to determine whether significant differences in serum hormone concentrations exist by stage and grade of disease.

Table1 . Median (25%, 75%) values of sex hormone-related factors according to prostate cancer status.

Hormone	Prostate Cancer (n=124)	Disease-free controls (n=406)	P-value*
Androstenedione (ng/ml)	6.28 (3.90, 9.06)	1.00 (0.80, 1.30)	**<0.0001**
Estradiol (pg/ml)	36.70 (26.75, 43.5)	28.90 (22.90, 36.40)	**<0.0001**
Estrone (ng/ml)	1.44 (1.04, 1.87)	1.91 (1.25, 2.94)	**<0.0001**
SHBG (nM)	42.15 (30.20, 56.60)	29.70 (21.50, 43.10)	**<0.0001**
Total testosterone (ng/dl)	445.50 (321.46, 611.62)	559.77 (413.88, 757.10)	**<0.0001**
IGF-1 (ng/ml)	233.66 (183.55, 288.16)	59.00 (41.00, 83.00)	**<0.0001**
IGFBP-1(ng/ml)	39.62 (31.89, 46.52)	3.32 (2.60, 4.33)	**<0.0001**

* Kruskal-Wallis Test.

CONCLUSIONS

Given the overall increased incidence of and mortality due to prostate cancer in African-American men, these men represent an appropriate population for the study of the associations between circulating hormones and genetic polymorphisms and prostate cancer risk. Our goal is to identify hormonal factors and genetic markers that can be used to stratify African-American men who are at risk for developing disease as well as those who progress to more severe disease.

Over the next 12 months, we will begin to analyze the hormone and genotype data with respect to prostate cancer risk. We will continue our strategy of selecting SNPs in candidate susceptibility genes to test for association with prostate cancer using the FMHS population. In the coming year, we will perform SNP discovery on one or more candidate genes to complement our knowledge of SNPs in the available public databases.

REFERENCES

(1) Sarma AV, Schottenfeld D. Prostate cancer incidence, mortality, and survival trends in the United States: 1981-2001. Semin Urol Oncol 2002; 20(1):3-9.